THE EFFECT OF CHOLINE ON PHOSPHOLIPIDE SYNTHESIS IN DOG LIVER SLICES*

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Various investigators have demonstrated that choline stimulates the formation of liver phospholipides in intact choline-deficient rats or dogs (1-3). The in vitro technique provided a critical test of the hypothesis that choline exerts this effect by direct action on the liver cell. With this procedure Artom and Swanson (4) showed that phospholipide synthesis is markedly depressed in liver slices from rats on a low protein diet and is not increased by supplementing this diet with choline. They also demonstrated that the addition of choline to choline-deficient slices in vitro stimulated phospholipide synthesis, though not to normal values. The present study is concerned with the quantitative relations between degrees of choline deficiency and liver phospholipide metabolism in vitro.

EXPERIMENTAL

Healthy, mongrel dogs maintained on a Purina chow diet served as control animals. The experimental dogs were fed 15 gm. per kilo of body weight of a high fat, low protein, choline-deficient diet (5) for periods extending from 7 to 21 days. Another group received the same diet with a 1 per cent choline chloride supplement1 for 21 days. Animals in a post-absorptive state were killed by rapid intracardiac air injection. A portion of the liver was quickly removed, washed in 0.9 per cent NaCl, blotted, sliced with a Martin slicer (6), and weighed on a microtorsion balance. One 0.5 mm. thick slice weighing 300 to 500 mg. was added to 5 ml. of calcium-free phosphate buffer (Medium II, type A (7)) of pH 7.3 to 7.4, containing 2.5 µc. of inorganic phosphate. The tissue and medium were aerated with O2 and shaken at a constant rate for a period of 1 hour at 37°. In each experiment 5 mg. of choline chloride were added to two or three samples, and an equal number of samples was incubated without choline. The time interval between sacrificing the dog and the beginning of incubation was ordinarily less than 7 minutes.

After incubation, the tissue and medium were transferred to a glass

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homogenizing tube containing 5 ml. of cold 20 per cent trichloroacetic acid (TCA). After homogenizing and centrifuging, the supernatant fluid was fractionated into inorganic and organic acid-soluble phosphates by magnesium precipitation (8). After two more washings with 10 per cent TCA, the lipides of the tissue residue were isolated by shaking twice with cold ethanol, extracting twice for 1 hour with ethanol at 60°, and an 8 hour Soxhlet extraction with boiling ethyl ether. The alcohol-ether extract was evaporated to near dryness under a vacuum, and reextracted with petroleum ether (8). As a result of the TCA treatment, the phosphorus concentration in the petroleum ether extract was about 20 per cent lower than the phospholipide phosphorus as determined by extraction of the fresh tissue with fat solvents. However, with the latter procedure the ether extracts contained significant amounts of non-phospholipide P\textsuperscript{32} which did not permit an accurate determination of phospholipide specific activity even after shaking with carrier phosphate. As a check on the TCA method several liver slices were incubated with radioactive phosphate for 10 seconds. Subsequent analysis showed that less than 0.01 per cent of the P\textsuperscript{32} was incorporated into the phospholipide fraction of these “zero time” livers. This amount of P\textsuperscript{32} was less than 5 per cent of the P\textsuperscript{32} in the phospholipide fraction after 1 hour incubation.

The choline-containing phospholipides were separated by adsorption on magnesium oxide and elution with methanol (9). Cephalin phosphorus was separated from the magnesium oxide by 15 hour heating at 70° with 1 N NaOH. Chemical and radioactive measurements of the phosphorus fractions of each slice were determined in duplicate as previously described (8).

Results

Preliminary work on the influence of Krebs-Henseleit’s Medium I (7), supplemented serum (7), and on the calcium-free phosphate medium on tissue phospholipide synthesis established the last medium as the medium of choice. The rate of phospholipide synthesis in the calcium-free phosphate buffer was equal to or greater than the rate in the other media. Calculation of the amount of the medium's inorganic phosphate converted to liver phospholipide was simplified by the relatively high concentration of phosphate in the calcium-free solution, which kept its specific activity at a constant level. The presence of choline in the medium did not affect the phospholipide concentration at the end of the 1 hour incubation period. In agreement with previous observations (10), the choline-deficient liver showed a progressive decrease in the concentration of choline-containing phospholipides (Table I); after 21 days of deficiency, the concentration was reduced to half the normal value, but the cephalin con-
centration, determined by the difference between total and choline-contain-
ing phospholipides, exhibited less of a decrease. The neutral fat con-
centration of the choline-deficient slices was increased, but not enough to account for the marked decrease of choline-containing phospholipides by dilution. Supplementing the high fat diet with 1 per cent choline chloride completely prevented fatty infiltration of the liver but only partially re-
stored the choline-containing phospholipide concentration.

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\begin{array}{cccccccc}
\text{Table I} \\
\text{Concentration* and Synthesis of Choline- and Non-Choline-Containing Phospholipides during 1 Hour's Incubation with and without Choline} \\
\hline
\text{Diet} & \text{Days} & \text{Dogs} & \text{Slices without choline} & \text{Slices with choline} & \text{Choline-containing phospholipide} & \text{Non-choline-containing phospholipide} & \text{P converted to choline-containing phospholipide in 1 hr.} & \text{P converted to non-choline-containing phospholipide in 1 hr.} \\
\hline
\text{Deficiency} & 0 & 4 & 10 & 10 & 0.67 & 0.31 & 7.6 & 8.3 \\
& 7 & 3 & 8 & 8 & ±0.02 & ±0.02 & 5.8 & 16.4 \\
& 16 & 4 & 11 & 11 & 0.38 & 0.26 & 4.4 & 14.5 \\
& 21 & 2 & 6 & 6 & ±0.02 & ±0.02 & 4.0 & 18.4 \\
\text{High fat + 1% choline chloride} & 21 & 4 & 12 & 12 & 0.44 & 0.31 & 3.7 & 4.7 \\
\end{array}
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* Concentrations are expressed as mg. of phospholipide phosphorus per gm. of fresh liver. Numerical values are given as the mean ± standard error.
† One slice per medium; each slice was analyzed separately.

As the concentration of choline-containing phospholipides decreased from 0.67 to 0.35 mg. of P per gm. with increasing periods of high fat, low protein feeding, the incorporation of phosphate into these phospholipides dimin-
ished from 7.6 to 4.0 γ per hour, a decrease of 47 per cent. No appreciable alteration was noted in the rate of synthesis of the cephalin fraction throughout the deficiency.

The addition of choline in vitro did not influence the rate of phospho-
lipide synthesis in normal liver slices, but in deficient slices it increased the rate of formation of choline-containing phospholipides even above that of normal slices. This finding is quantitatively different from that of Artom and Swanson (4), who observed that in the rat the addition of choline to
choline-deficient slices only partially restored the rate of phospholipide synthesis. This stimulatory effect increased progressively with time on the high fat diet. The synthesis of choline-containing phospholipides increased 183, 230, and 360 per cent at the 7th, 16th, and 21st day of deficiency, respectively. The addition of 1 per cent choline chloride to the high fat diet for a period of 3 weeks did not improve the rate of synthesis of choline-containing phospholipides but prevented a significant stimulation of synthesis by the addition of choline in vitro. Apparently, the degree of stimulation in vitro is a better test for choline deficiency than the absolute decrease in synthesis rate.

DISCUSSION

The formation of phospholipides by surviving liver slices has been studied in rats by Fishler et al. (11), Taurog et al. (12), and Artom and Swanson (4). Recalculation of their results showed that the normal rat liver converted 1.4 to 4.9 γ of phosphorus to phospholipide per gm. of liver per hour. This is considerably less than a conversion rate of 10.4 to 15.7 γ per gm. per hour that we observed in normal dog liver slices. This difference, as well as other differences between our present results on dogs and those obtained by others on rat liver slices, may be due to a difference in species, diet, or technique.

Artom and Swanson have interpreted the decreased synthesis of phospholipides in slices of choline-deficient rats as due chiefly to a decrease in cellular activity resulting from a protein deficiency. The finding that supplementing the high fat, low protein diet with 1 per cent choline chloride did not restore the ability of the dog liver slice to synthesize choline-containing phospholipides appears to support their contention. A greater rôle could, however, be attributed to a choline deficiency, as indicated by our findings that non-choline-containing phospholipides and acid-soluble phosphates were synthesized at normal rates in the choline-deficient dog liver slices, and that the addition of choline to the choline-deficient slice increased the synthesis of choline-containing phospholipides to values above normal.

Platt and Porter (13) have suggested that the stimulation of phospholipide synthesis by choline in the intact rat is the result of a mass action effect. This hypothesis does not explain why the addition of excess choline to normal liver slices of dogs as well as rats (4) does not increase phospholipide synthesis.

The stimulatory effect of choline on the synthesis of phospholipides in vitro is very similar to the action of choline in the intact dog (3), rat (1),

*Percentage increase on the 7th day of deficiency is equal to 100 × ((16.4 - 5.8)/-5.8) = 183.
and man (14). This suggests that choline, in vivo as well as in vitro, exerts a direct action on the liver cell. The finding that the magnitude of the stimulatory effect depends upon the severity of the choline-deficient state might provide a quantitative measure of choline deficiency. This in vitro procedure, coupled with the liver biopsy technique, might possibly be employed to assay the severity of choline deficiency in patients and to test the efficacy of treatment with lipotropic agents.

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SUMMARY

The dog maintained on a high fat, low protein, choline-deficient diet is characterized by a progressive decrease in the concentration of choline-containing phospholipides in the liver and a diminished ability of the liver slice to synthesize these phospholipides. The addition of choline to the medium had no effect on the rate of phospholipide synthesis of the normal slice, or a slice obtained from a dog maintained on the deficient diet receiving a choline supplement.

The addition of choline to the choline-deficient liver slice markedly increased the synthesis of choline-containing phospholipides. The degree of stimulation by choline in vitro increased with the severity of the deficiency. The synthesis of non-choline-containing phospholipides was not affected by choline deficiency or the addition of choline in vitro.

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